

Method for obtaining three products with different properties from fennel (*Foeniculum vulgare*) seed[☆]

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ARTICLE INFO

Article history:

Received 15 April 2014

Received in revised form 10 June 2014

Accepted 11 June 2014

Keywords:

Distillation

Essential oil

Foeniculum vulgare

Lipid

Steam distillation

ABSTRACT

The objectives of this study were to determine the effects of distillation time (DT; 15–1080 min) on yield, composition, and antioxidant capacity of fennel (*Foeniculum vulgare*) seed essential oil (EO) as well as on the yield, composition, and properties of lipids extracted from steam-distilled fennel seeds (15–600 min). EO yield increased with increasing DT to a maximum of 1.375% at 1080 min. The principal constituent was estragole, comprising 82–91% of the overall content. Other species included limonene, fenchone, and anethole. Antioxidant capacity of the EO was essentially unaffected by DT, with capacities ranging from 11.2 to 20.6 µmol Trolox/g. The yield of lipids extracted from steam distilled fennel seeds was unaffected by DT and ranged from 21.7 to 22.8 mass%. The fatty acid composition was also unaffected by DT, and the major constituents were petroselenic (67.0–71.3%) and oleic (12.0–16.4%) acids. The concentrations of tocopherols, tocotrienols, and phytosterols were unaffected by DT whereas unsaponifiables and EO content in lipids decreased with increasing DT. Acid value, kinematic viscosity, peroxide value, and pour point increased with increasing DT, whereas density decreased. Induction period, heteroatom content, and Gardner color were unaffected by DT. As DT increased, *in vitro* degradability of defatted, steam-distilled fennel seeds decreased. In summary, longer DT negatively impacted feed quality of steam-distilled, defatted seed meal and lipid quality but did not significantly affect EO composition and antioxidant capacity.

Published by Elsevier B.V.

1. Introduction

Essential oils (EOs) are volatile secondary metabolites with applications in perfumes, cosmetics, soaps, incense, aromatherapy, food and beverage flavorings, and household cleaning products. EOs are also noteworthy for antioxidant and biological activities, including as bactericidal, virucidal, fungicidal, antiparasitical, and

insecticidal agents (Bakkali et al., 2008; Bozin et al., 2006; Daferera et al., 2000; Dapkevicius et al., 1998; Lis-Balchin et al., 1998; Nerio et al., 2010). Notable examples of commercialized EOs include those from eucalyptus (*Eucalyptus globulus*), lavender (genus *Lavandula*) and fennel (*Foeniculum vulgare*) (Lis-Balchin et al., 1998; Nerio et al., 2010). The most common industrial methods for isolation of EOs are steam distillation (SD) and hydrodistillation (Hawthorne et al., 1993; Jimenez-Carmona et al., 1999; Luque de Castro et al., 1999; Smith, 2002). Other methods include extraction with organic solvents, supercritical CO₂ and superheated (subcritical) water as well as ultrasound and microwave-assisted extraction (Dapkevicius et al., 1998; Hawthorne et al., 1993; Jimenez-Carmona et al., 1999; Lucchesi et al., 2004; Luque de Castro et al., 1999).

Several EOs are found in lipid-bearing seeds from the Apiaceae family, including anise (*Pimpinella anisum*), coriander (*Coriandrum*

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sativum), cumin (*Cuminum cyminum*), and fennel (*F. vulgare*) (Damjanovic et al., 2005; Eikani et al., 1999; Illes et al., 2000; Shokri et al., 2011). Fennel EO (FEO) is extracted either from seeds or biomass and each has a distinct chemical profile and uses. Previous studies demonstrated that distillation time (DT) can significantly affect yield and composition of EOs from pine (*Pinus ponderosa* Dougl. ex Laws), female and male Rocky Mountain juniper (*Juniperus scopulorum* Sarg.), peppermint (*Mentha x piperita* L.), lemongrass (*Cymbopogon flexuosus* Steud.), palmarosa (*Cymbopogon martinii* Roxb.), Japanese cormmint (*Mentha canadensis* L.), oregano (*Origanum vulgare* L.), and fennel herb (Cannon et al., 2013; Zheljazkov and Astatkie, 2012; Zheljazkov et al., 2012a,b, 2013a,b). While the effect of DT on fennel herb EO was reported (Zheljazkov et al., 2013b), there is no information on how DT may impact the quality of lipids extracted from fennel seeds.

Fennel seeds contain lipids in addition to EO. The principal fatty acid (FA) in fennel seed oil (FSO) comprising 60–85% of the content is petroselenic acid (PA; C18:1 6c; 6Z-octadecenoic acid) (Charvet et al., 1991; Placek, 1963). PA is of particular interest because its oxidation yields lauric and adipic acids (Corma et al., 2007; Placek, 1963). Lauric acid is used in the soap, cosmetic, medical, and perfume industries whereas adipic acid is a precursor to industrially significant polymers (Wittcoff et al., 2004). PA represents a renewable route to bio-based adipic acid, thus obviating the petrochemical route to adipic acid. Consequently, EOs, FAs including PA and seed meal may be obtained from fennel seeds, all of which have industrial utility. Other uses for FSO may include biodiesel, as a previous study reported favorable fuel properties of FA methyl esters (FAMEs) from PA-containing coriander seed oil (Moser and Vaughn, 2010). Another study demonstrated that biodiesel fuels high in monounsaturated FAs yields superior fuel properties relative to those enriched in saturated or polyunsaturated FAs (Moser and Vaughn, 2012). Monounsaturated FAs may comprise 86% of FA profile of FSO (Placek, 1963).

Our objectives were to measure the influence of DT on composition of FEO, determine the influence of DT on quality of lipids extracted from fennel seeds, and assess the composition of the resulting seed meal. Fennel seeds were initially subjected to DT ranging from 15 to 1080 min in duration. Lipids were solvent-extracted from the resulting steam-distilled seeds, which were then characterized for their physical and chemical properties. The seed meal resulting from lipid extraction was also characterized for composition and *in vitro* degradability to ascertain its suitability as an animal feed.

2. Materials and methods

2.1. Materials

FAME standards (>99%) were purchased from Nu-Chek Prep, Inc. (Elysian, MN). (R)-(+)-limonene and estragole were obtained from Fluka (Buchs, Switzerland). Single element calibration standards were obtained from SCP Science (Champlain, NY). All other reagents and standards were purchased from Sigma-Aldrich Corp. (St. Louis, MO) and used as received.

2.2. Steam distillation of fennel seeds to remove essential oil component

SD of fennel seed (750 g) was conducted in triplicate in 2-L SD units (Hearthmagic, Rancho Santa Fe, CA), as described previously by Gawde et al. (2009). The DT tested were 15, 30, 60, 120, 240, 360, 480, 600, 720, 840, 960, and 1080 min. DT was measured from the deposition of the first drop of EO in the Florentina separator. The extracted EOs were collected at the end of each DT, the EO was

separated from the water and its weight determined. The samples were then stored at -5 °C. FEO yield was calculated as percentage of fennel seed weight.

2.3. Essential oil quantitative analysis by GC-FID

EO samples were analyzed by GC-FID on a Varian CP-3800 GC equipped with a DB-5 fused silica capillary column (30 m × 0.25 mm; 0.25 μm film thickness) operated using the following conditions: injector temperature, 240 °C; column temperature, 60–120 at 3 °C/min, then held at 240 °C at 20 °C/min for 5 min; carrier gas, He; injection volume, 1 μL (split on FID, split ratio 50:1); FID temperature was 300 °C.

With eight concentration points, a least squares regression for quantification was used. Each specific analyte was used to formulate a separate calibration curve using FID response data. Linearity was imposed by using response factors (RF) and regression coefficients independently. Response factors (RF) were calculated using the equation $RF = DR/C$, where DR was the detector response in signal area (SA) and C was the analyte concentration. The chromatograms of each of the EO samples from the field experiments were compared to the chromatograms from standards. Target analytes were confirmed by retention time. Confirmed integrated peaks were used to determine percentage of each chemical constituent in the EO itself. The RF of the target chemical constituent was used to determine the individual analyte percentage for each sample using the equation $SA/RF/C \times 100 = \% \text{ analyte in EO}$ on a wt (analyte)/wt (oil) basis.

2.4. Extraction of lipids from steam-distilled fennel seeds

The lipid content of the steam-distilled seeds was determined using a pulsed NMR spectrometer (Minispec, Bruker Optics Inc., Billerica, MA). Aliquots of FSO were used to calibrate the spectrometer. Steam-distilled fennel seeds were extracted with hexane for 6 h utilizing a conventional Soxhlet apparatus. Hexane was removed by rotary evaporation (10 mbar, 25 °C). The extracted oil was then filtered through Celite 545 (Advanced Minerals Corp., Santa Barbara, CA) to remove the remaining fine particulates suspended in the oil.

2.5. Fatty acid composition

Derivatization to FAMEs (2 N methanolic KOH) was performed as described previously (Ichihara et al., 1996) and analyzed using a PerkinElmer (Waltham, MA) Clarus 580 GC equipped with an FID, a built-in auto-sampler and an HP88 column (30 m × 0.25 mm; 0.20 μm film thickness). The carrier gas was H₂ with a flow rate of 15.0 mL/min. The temperature program was: hold at 100 °C for 5 min, ramp from 100 °C to 220 °C at 10 °C/min and hold at 220 °C for 15 min. Injection volume was 1.0 μL with a split ratio of 10.0:1. The injector and detector temperatures were 240 °C and 280 °C, respectively. FAME peaks were identified (triplicates, means reported) by comparison to reference standards.

2.6. Measurement of essential oil content in lipid fraction

Hexane extracts after Soxhlet extraction (1.8 mL) were derivatized to FAMEs (2 N methanolic KOH; 200 μL) and separated via GC-FID as described in Section 2.4. Estragole with a retention time of 5.13 min was used as a marker for essential oil content in the lipid fraction. The response factor of estragole with methyl oleate (99.9%) as an internal standard was 0.9921. Analyses were performed in triplicate with mean values reported.

2.7. Quantification of tocopherols, tocotrienols and phytosterols in lipid fraction

FSO samples were dissolved in hexane at a concentration of 10 mg/mL. Samples were injected in duplicate via autosampler onto a Varian ProStar HPLC (Agilent Technologies, Santa Clara, CA) fitted with an Inertsil (Varian Associates, Walnut Creek, CA) silica column (5 µm, 150 Å, 250 mm × 3.0 mm). Compounds were detected with a Varian Prostar model 363 fluorescence detector set at 290 nm for excitation and 330 nm for emission. The mobile phase consisted of hexane:THF (97:3 vol/vol) and was used at a flow rate of 1 mL/min. Tocopherols were identified by comparison to retention times of reference standards. Quantification of tocopherols and tocotrienols was performed using external standard calibration.

FSO samples (25 mg) with 5- α -cholestane added as an internal standard were saponified in 2 N ethanolic KOH at 60 °C for 1 h. Unsaponifiable material was extracted with hexane, which was evaporated under N₂. Trimethylsilyl (TMS) derivatives were prepared by adding 100 µL each of pyridine and BSTFA + 1% TMCS and subsequently heating at 60 °C for 45–60 min. Samples were then dried under N₂ and suspended in 200 µL CHCl₃. Samples (1 µL) were injected into a Varian 3800 GC equipped with a Supelco DB-5 (30 m × 0.25 mm; 0.25 µm film thickness) capillary column and FID. The carrier gas was He with a 1:50 injector split. The injector and detector temperatures were 270 and 280 °C, respectively. The temperature program was maintained at 250 °C for 0.5 min, increased at 10 °C/min to 270 °C, held for 27 min, then increased at 10 °C/min to 280 °C, and finally held for a further 3.5 min. Phytosterols were identified by comparison to retention times of reference standards. Phytosterols without available standards were identified by their relative retention times compared to the literature. Quantification (in triplicate) was carried out by the internal standard method.

2.8. Properties of lipid fraction

Unless specified otherwise, properties were measured in triplicate ($n=3$) with mean values reported following American Oil Chemists' Society (AOCS), American Society for Testing and Materials (ASTM), and European Committee for Standardization (CEN) standard test methods using instrumentation described previously (Moser et al., 2009; Moser and Vaughn, 2010, 2012): acid value (AV, mg KOH/g), AOCS Cd 3d-63; density (25 °C, g/mL), ASTM D4052; Gardner color (single determination), AOCS Td 1a-64; induction period (IP, 110 °C, h), EN 15751; kinematic viscosity (KV, 40 °C, mm²/s), ASTM D445; pour point (PP, °C), ASTM D5949; unsaponifiable matter (mg/g), AOCS Ca 6b-53. For a greater degree of precision, PP was measured with a resolution of 1 °C instead of the specified 3 °C increment.

Peroxide value (PV) was determined using the IDF method of Shantha and Decker (1994). Briefly, 10 mg of sample was added to chloroform:methanol (9.8 mL; 7:3 v/v) and the mixture was agitated for 2–4 s. Ammonium thiocyanate (50 µL; 3.94 M solution) was then added, followed by addition of ferrous chloride (50 µL; 0.018 M) with agitation for a further 2–4 s. Absorbance at 500 nm was measured on a Perkin-Elmer Lambda 35 spectrophotometer after the sample was equilibrated to room temperature (5 min) and transferred into a quartz cuvette. PVs were calculated from a standard curve of ferric chloride as described by Shantha and Decker (1994) and are expressed as milliequivalents (meq) peroxide/kg oil.

Lipid samples were subjected to microwave-assisted nitric acid digestion prior to Na, K, Mg, and Ca analyses. Samples were placed in digestion vessels (3 MPa max) and 10 mL of 67–70% nitric acid was added. Digestion was accomplished in a 1200 W microwave oven (Milestone Ethos EX, Milestone Inc., Shelton, CT) according

to the following program: ramp to 170 °C for 15 min followed by a 10 min hold at 170 °C. After cooling, the digests were diluted in deionized water. Elemental analyses were performed using inductively coupled plasma optical emission spectroscopy (PerkinElmer Optima 7000DV, PerkinElmer Inc., Waltham, MA) following EN 14538 (Na, K, Mg, and Ca), ASTM D4951 (P), and ASTM D5453 (S).

2.9. Essential oil antioxidant capacity

Antioxidant activity of FEO was measured in triplicate using the ORAC_{Oil} (oxygen radical absorbance capacity in bulk oil) method (Huang et al., 2002a, 2002b) with Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) as the standard and is expressed as µmol Trolox/g.

2.10. Forage quality of the double extracted fennel seed

Chemical analysis of steam-distilled, defatted fennel seeds was performed by Cumberland Valley Analytical Services (Maugansville, MD) using wet chemistry methods. Concentrations of non-fibrous carbohydrates (NFC) and net energy for lactation (NEL) were estimated based on chemical composition. *In vitro* dry matter degradability (DMD) was analyzed using ruminal inoculum. The procedures used for these analyses are available at: <http://www.foragelab.com/Resources/Lab-Procedures/>; accessed December 5, 2013).

2.11. Statistical analysis

The effect of DT on overall EO yield (%), concentration (%) and yield (mg/100 g seed) of limonene, fenchone, estragole, anethole, 17 nutrients [dry matter (DM), crude protein (CP), soluble protein (% CP), soluble protein (% DM), acid detergent fiber (ADF; % DM), neutral detergent fiber (NDF; % DM), DMD, ash (% DM), Ca (% DM), P (% DM), Mg (% DM), K (% DM), Na (% DM), Fe (ppm), Mn (ppm), Zn (ppm), and Cu (ppm)], and antioxidant capacity was determined using one-way analysis of variance (ANOVA). In addition, one-way ANOVA was employed to determine the effect of DT on yield of FSO, content of FEO, unsaponifiables and heteroatoms in FSO, as well as the effect of DT on AV, density, KV, PP, and PV of FSO. The validity of model assumptions was verified by examining the residuals as described in Montgomery (2013). For responses where the effect of DT was significant (p -value <0.05), multiple means comparison was completed using Duncan's multiple range test at the 5% level of significance, and letter groupings were generated. The analyses were completed using the GLM Procedure of SAS (SAS Institute Inc., 2010).

The relationships between DT and concentration and yield of EO constituents are described by the Michaelis–Menton (Eq. (1)), Power (Eq. (2)), and polynomial (Eq. (3)) models. The parameters of the nonlinear models were estimated iteratively using the NLIN Procedure (SAS Institute Inc., 2010), and the fitted models met all adequacy requirements (Bates and Watts, 2007).

$$Y = \frac{\theta_1 X}{\theta_2 + X} + \varepsilon \quad (1)$$

$$Y = \theta_1 X^{\theta_2} + \varepsilon \quad (2)$$

$$Y = \beta_0 + \beta_1 X + \beta_2 X^2 + \beta_3 X^3 + \varepsilon \quad (3)$$

Y is the dependent (response) variable, X is the independent (DT) variable, and the error term ε is assumed to have normal distribution with constant variance. Validity of the normality, constant variance and independence assumptions on the error terms were verified by examining the residuals (Bates and Watts, 2007).

Table 1

Mean FEO yield (%) along with the concentration (C; %) and yield (Y; mg/100 g seed) of limonene, fenchone, estragole, and anethole, and antioxidant capacity ($\mu\text{mol Trolox/g}$) of FEO at eight distillation times (DT).^a

DT (min)	FEO yield	Limonene C	Fenchone C	Estragole C	Anethole C	Limonene Y	Fenchone Y	Estragole Y	Anethole Y	Antioxidant Capacity
15	0.042 i	2.74 l	3.50 bcd	89 ab	1.017 a	1.1 l	1.5 h	37 i	0.43 i	13.9 b
30	0.072 i	3.48 k	3.34 cde	90 ab	0.997 ab	2.5 k	2.4 g	65 i	0.72 i	14.0 b
60	0.157 h	4.13 j	3.36 cde	91 a	0.953 bc	6.5 j	5.3 f	142 h	1.49 h	13.3 b
120	0.276 g	4.54 i	3.12 e	88 ab	0.937 c	12.5 i	8.6 e	243 g	2.58 g	12.1 b
240	0.573 f	6.19 h	3.65 b	89 ab	0.843 d	35.5 h	20.9 d	508 f	4.85 f	11.2 b
360	0.782 e	6.66 g	3.46 bcd	87 bc	0.843 d	52.1 g	27.1 c	680 e	6.61 e	15.0 b
480	0.923 d	7.22 f	3.93 a	90 ab	0.857 d	66.7 f	36.3 b	828 d	7.91 d	14.3 b
600	1.030 c	7.57 e	3.64 b	88 ab	0.857 d	78.0 e	37.4 b	907 c	8.81 b	11.2 b
720	1.053 c	8.30 d	3.53 bc	86 bc	0.815 d	87.4 d	37.1 b	910 c	8.59 bc	14.5 b
840	1.201 b	8.64 c	3.60 bc	84 cd	0.820 d	104 c	43.3 a	1014 b	9.85 a	14.2 b
960	1.353 a	10.12 b	3.25 de	82 d	0.600 e	137 b	44.0 a	1111 a	8.13 cd	15.6 b
1080	1.375 a	10.66 a	3.17 e	83 d	0.603 e	147 a	43.5 a	1137 a	8.31 bcd	20.6 a

^a Mean values within a column sharing the same letter are not significantly different based on Duncan's multiple range test at the 5% level of significance.

3. Results and discussion

3.1. Effect of DT on FEO yield and composition

As depicted in **Table 1**, DT affected yield of FEO from seeds. FEO yield increased from 0.042 wt% at 15 min with increasing DT and reached a maximum of 1.375% at 1080 min. Nearly all DTs beyond 30 min provided FEO yields statistically different from the others. The yields at DT = 15 and 30 min, 600 and 720 min, and 960 and 1080 min were equivalent.

The major constituents identified in FEO were estragole, fenchone, limonene, and anethole, as seen in **Table 1**. The most abundant species was estragole (methyl chavicol), which comprised 82–91% of the overall EO content. DT had little effect on estragole concentration up to 600 min, as statistically equivalent amounts were measured at nearly all DTs. Only DT = 60 (91%) and 360 min (87%) yielded statistically different results below 720 min. Beyond 600 min statistically significant decreases in estragole concentration were noted, as the DT = 960 and 1080 min samples exhibited the lowest values at 82 and 83%, respectively.

The concentration of fenchone ranged from 3.12 (120 min) to 3.93% (480 min). Several DTs yielded statistically equivalent results, but no trend in fenchone concentration versus DT was elucidated. For instance, fenchone concentrations at 15, 240, 360, 600, 720, and 840 min were statistically equivalent whereas 30, 60, 120, 960, and 1080 min yielded equivalent results.

Higher DTs afforded statistically significant higher concentrations of limonene. The concentrations of limonene at 15 and 1080 min were 2.74 and 10.66%, respectively, resulting in a 3.89-fold increase from 15 to 1080 min. This magnitude of increase was higher than those calculated for fenchone (0.91), estragole (0.93), and anethole (0.59). Paradoxically, limonene had the lowest boiling point (bp) of these constituents, yet its concentration increased with higher DTs. For comparison, the bps of limonene, fenchone,

estragole, and anethole are 176, 194, 216, and 234 °C, respectively (Lide, 2008). Intuitively, the opposite behavior was expected. We speculate that perhaps limonene was not as readily transportable to the seed surface for extraction relative to the other analytes.

The influence of DT on anethole was opposite to that observed for limonene. Specifically, longer DTs resulted in statistically significant lower concentrations of anethole. For example, the concentration of anethole at DT = 15 min was 1.017% whereas at 960–1080 min it was statistically equivalent and ranged from 0.600% to 0.603%. Anethole was the least volatile of the species examined in this study, so we speculate that it was least affected by DT. The rate of distillation for the other constituents was probably higher than for anethole, thus resulting in lower observed anethole concentrations with increasing DT relative to the other species.

Yields of estragole, fenchone, limonene, and anethole were calculated from EO yield and the concentrations of individual constituents at each DT and are expressed as mg/100 g seed. As expected, the yield of individual EO species was lower at DTs of 15 or 30 min and increased as DT was increased. For example, limonene increased from 1.1 mg/100 g at DT = 15 min to 147 mg/100 g at 1080 min. Limonene, fenchone, and estragole reached their maximum yields at DT = 1080 min. However, anethole reached its maximum at 840 min and decreased thereafter. In most cases, the incremental increases in yield as DT increased were statistically significant.

As seen in **Fig. 1**, the relationships between DT and EO concentration, as well as the yields of fenchone, estragole, and anethole are described by the Michaelis–Menten model (Eq. (1)). The relationships between DT and concentration and yield of limonene are described by the Power model (Eq. (2)). The relationships between DT and the concentrations of estragole and anethole are described by the second and third order polynomial models (Eq. (3)), respectively. While the polynomial model was linear, the other models were nonlinear.

Table 2

Mean soluble protein expressed as weight percentages of crude protein (% CP) and dry matter (% DM) as well as the weight percentages (% DM) of ADF, NDF, ash, K, and Na in steam distilled, defatted fennel seed meal.^a

DT (min)	DM (%)	Soluble protein (% CP)	Soluble protein (% DM)	ADF (% DM)	NDF (% DM)	Ash (% DM)	K (% DM)	Na (% DM)	DMD (%)
15	95.0 a	25.4 a	6.17 a	24.4 d	43.0 d	9.78 a	2.93 a	0.114 ab	70.5 a
30	94.6 abc	24.1 a	5.83 a	24.4 d	42.5 d	10.02 a	3.02 a	0.118 a	69.7 a
60	94.9 ab	22.0 b	5.30 b	24.4 d	44.0 d	10.04 a	3.02 a	0.116 ab	70.8 a
120	94.5 bcd	21.5 b	5.13 b	25.3 cd	43.7 d	9.77 a	3.01 a	0.115 ab	68.4 a
240	94.4 cd	18.3 c	4.37 c	27.0 c	47.5 c	9.88 a	2.87 ab	0.111 bc	62.9 b
360	94.1 d	17.0 c	4.13 c	29.1 b	51.2 b	9.50 b	2.72 bc	0.107 cd	61.5 b
480	94.4 cd	17.5 c	4.20 c	30.0 b	51.1 b	9.07 c	2.62 c	0.107 cd	62.1 b
600	94.3 cd	17.1 c	4.20 c	33.3 a	53.5 a	8.93 c	2.46 d	0.102 d	61.1 b

ADF = acid detergent fiber; CP = crude protein; DM = dry matter; DMD = dry matter degradability; DT = distillation time; K = potassium; Na = sodium; NDF = neutral detergent fiber.

^a Mean values within a column sharing the same letter are not significantly different based on Duncan's multiple range test at the 5% level of significance.

3.2. Effect of DT on EO antioxidant activity

The antioxidant capacity of FEO as determined by the ORAC method is presented in Table 1. The capacities ranged from 11.2 $\mu\text{mol Trolox/g}$ at 240 and 600 min to 20.6 $\mu\text{mol Trolox/g}$ at 1080 min. However, all DTs yielded antioxidant capacities that were statistically equivalent (11.2–15.6 $\mu\text{mol Trolox/g}$) with the exception of DT = 1080 min. Therefore, these results indicate that DT up to 960 min had no appreciable effect on antioxidant capacity of FEO. We speculate that the increased capacity at 1080 min was due to a subtle change in FEO profile that resulted in increased capacity to scavenge free radicals. The cause of the increase in capacity at 1080 min requires further investigation beyond the scope of the current study.

3.3. Effect of DT on composition of defatted fennel seed meal

The composition of steam distilled, defatted fennel seed meal is presented in Table 2. Analysis was only possible up to a DT of 600 min due to insufficient sample size at DTs of 720 min and beyond. The concentrations of soluble nutrients (soluble protein,

Na, and K) decreased or tended to decrease with increasing DT. For instance, soluble protein as a percentage of crude protein decreased from 25.4% at DT = 15 min to 17.1% at 600 min. Correspondingly, insoluble matter (NDF and ADF) increased with increasing DT. The changes in concentration noted for soluble protein, K, Na, ADF, and NDF were statistically significant over the course of 600 min of SD (Table 2). However, the concentrations of Ca, P, Mg, Fe, Mn, Zn, and Cu were not significantly affected ($P > 0.05$) by DT.

3.4. Effect of DT on in vitro degradability of defatted fennel seed meal

In vitro DMD data, an indication of the energy value of the feed, are shown in Table 2. The procedure is designed to simulate *in vivo* rumen fermentation. Statistically equivalent DMD were obtained for DT = 15–120 min. A second set of statistically equivalent DMD were observed for DTs > 120 min. Thus, *in vitro* DMD of defatted, steam-distilled fennel seed meal was reduced by 9% with DTs > 120 min. The causes of reduced degradability were lower contents of readily degradable nutrients coupled with higher concentrations of indigestible fiber fractions with increasing DT. These

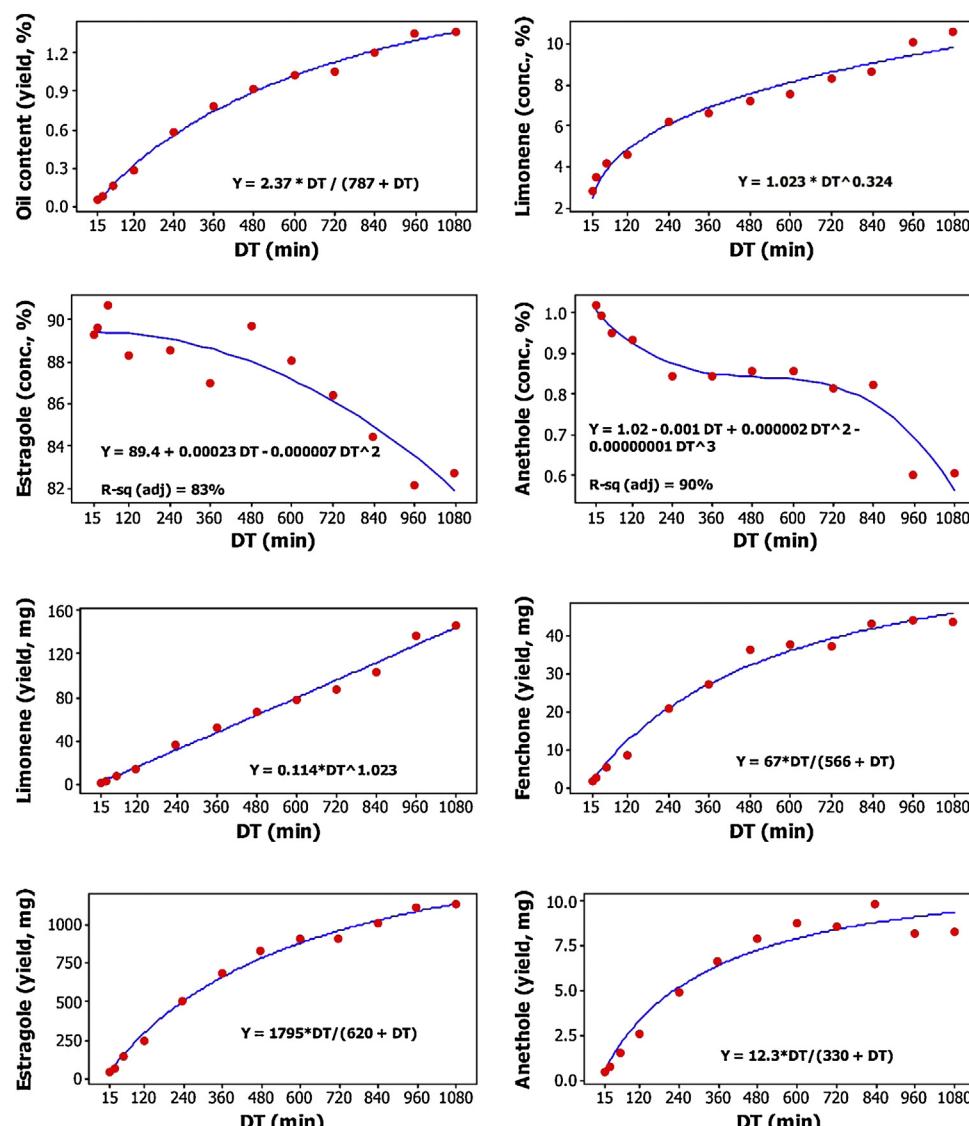


Fig. 1. Plot of DT vs EO yield (%), the concentration (%) of limonene and anethole, and the yields (mg/100 g seed) of limonene, fenchone, estragole and anethole along with the fitted (solid line) Michaelis-Menten, power and polynomial models.

Table 3

Lipid content of steam distilled fennel seeds along with a summary of the distribution of non-lipid constituents in the resulting lipid fractions.^a

DT (min)	FSO (mass%)	FEO (mass%) ^b	Tocopherols (ppm) ^c	Tocotrienols (ppm) ^c	Phytosterols (ppm) ^c	Unsaponifiables (mass%)	Heteroatoms (ppm) ^d
Control	22.5 ab	0.81 a	12.9	296.6	6340	7.62 ab	700
15	21.7 b	0.81 a	9.9	345.6	6473	7.83 a	515
30	21.9 b	0.79 a	12.4	374.9	6401	7.84 ab	554
60	21.9 b	0.68 b	9.7	326.6	6125	7.27 b	493
120	21.9 b	0.62 c	10.7	320.6	6612	6.63 bc	506
240	22.3 a	0.42 d	13.3	301.8	6999	4.89 cd	684
360	22.3 a	0.34 e	13.8	286.4	7271	4.41 d	606
480	22.1 a	0.25 f	19.9	327.9	7582	4.53 d	588
600	22.8 ab	0.18 g	15.0	282.2	6876	3.90 d	450

FEO = fennel essential oil; FSO = fennel seed oil (lipids).

^a Mean values within a column sharing the same letter are not significantly different based on Duncan's multiple range test at the 5% level of significance.

^b Determined by converting mg/g Estragole (**Table 4**) into mass% and dividing by the weight fraction of estragole in fennel EO (**Table 1**).

^c Summarized from **Table 6**.

^d Summarized from **Table 5**.

Table 4

Fatty acid profile (area %) of lipid fractions obtained after hexane extraction of steam-distilled fennel seeds.^{a,b} Also indicated is the concentration of estragole measured in lipid fractions as a marker for FEO content.

DT (min)	C16:0	C16:1 9c	C18:0	C18:1 6c	C18:1 9c	C18:2 9c, 12c	C18:3 6c, 9c, 12c	C18:3 9c, 12c, 15c	C20:0	Unknown (sum)	Estragole (mg/g)
Control	4.1 (0.1)	0.4	1.1	69.2 (2.3)	13.9 (2.1)	10.0 (0.2)	0.2	0.2	0.3	0.7 (0.1)	7.24
15	4.0	0.4	1.2	67.8 (1.9)	15.1 (1.7)	10.1 (0.1)	0.2	0.2	0.3	0.8 (0.1)	7.20
30	4.0	0.4	1.1	67.0 (1.2)	16.4 (1.3)	10.0 (0.1)	0.2	0.2	0.3	0.6 (0.1)	7.08
60	4.0	0.4	1.1	67.6 (1.4)	15.8 (0.1)	10.0	0.2	0.2	0.3	0.4 (0.1)	6.21
120	4.0	0.4	1.2	68.5 (1.2)	14.7 (1.2)	10.1	0.2	0.2	0.3	0.4 (0.1)	5.48
240	4.1	0.4	1.2	68.5 (2.2)	14.8 (2.1)	9.9 (0.1)	0.2	0.2	0.3	0.5 (0.1)	3.76
360	4.1	0.4	1.1	68.5 (1.8)	14.7 (1.8)	10.0 (0.1)	0.2	0.2	0.3	0.5 (0.1)	2.94
480	4.1	0.4	1.2	70.1 (2.8)	12.8 (2.7)	10.2	0.2	0.2	0.3	0.5 (0.1)	2.28
600	4.1	0.4	1.1 (0.1)	71.3 (1.5)	12.0 (1.5)	10.1 (0.1)	0.2	0.2	0.3	0.4 (0.1)	1.61

^a C18:1 9c signifies an 18 carbon FA with one cis (c) double bond located at C9 (methyl 9Z-octadecenoate; methyl oleate).

^b Numbers in parenthesis represent standard deviations from the reported means ($n=3$). Where none is indicated the standard deviation was zero.

changes corresponded to decreased animal feeding value above DT = 120 min.

3.5. Effect of DT on lipid composition

Depicted in **Table 3** is the lipid content of steam distilled fennel seeds along with the overall concentrations of FEO, tocopherols, tocotrienols, phytosterols, unsaponifiable matter, and heteroatoms in FSO. The FA profile of FSO along with the concentration of estragole as a marker for FEO content are presented in **Table 4**. The concentrations of specific heteroatoms such as Na, K, Ca, Mg, S, and P are displayed in **Table 5**. The concentrations of individual tocopherols, tocotrienols, and phytosterols are depicted in **Table 6**. Data for samples beyond DT = 600 min were not available due to insufficient sample size.

Undistilled fennel seeds (control) contained 22.5 mass% FSO (lipids), 0.81 mass% FEO, and 7.62 mass% unsaponifiables. SD had minimal impact on lipid content, as the content of lipids in the control sample (DT = 0 min) was statistically equivalent to those obtained for DT = 15 through 600 min (21.7–22.8 mass%). Such a result was not surprising, as SD was not expected to remove FSO

from the seeds. As expected, the content of FEO in FSO decreased significantly with increasing DT. For instance, the concentrations of FEO at DT = 15 and 600 min were 0.81 and 0.18 mass%, respectively. Presumably, DTs > 600 min would extract the remainder of FEO from intact seeds, thus further reducing their concentration in FSO. The content of unsaponifiable matter in FSO decreased significantly with increasing DT, which was not surprising since unsaponifiables include FEO constituents along with other compounds such as tocopherols, tocotrienols, and phytosterols. At DT = 600 min the concentration of unsaponifiables in FSO had decreased to 3.90 mass% from 7.83 mass% at 15 min. Correspondingly, the concentration of estragole decreased from 7.24 mg/g (control) to 1.61 mg/g at 600 min.

FA composition was unaffected by DT. All samples contained 4.0–4.1% palmitic acid (C16:0), 0.4% palmitoleic acid (C16:1), 1.1–1.2% stearic acid (C18:0), 9.9–10.1% linoleic acid (C18:2), 0.2% α -linolenic acid, 0.2% γ -linolenic acid, and 0.3% behenic acid (C20:0). The content of PA (C18:1 6c) ranged from 67.0% at DT = 30 min to 71.3% at 600 min whereas oleic acid (C18:1 9c) was present in concentrations ranging from 12.0% (600 min) to 16.4% (30 min).

Table 5

Heteroatom content of lipid fractions obtained after hexane extraction of steam-distilled fennel seeds.^a

DT (min)	S (ppm)	P (ppm)	Na (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)
Control	49 ab	323 abc	23 ab	88 abcd	137 abcd	80 a
15	11 bc	267 bcd	10 bc	69 bcdef	106 def	52 cde
30	8 bc	284 abc	16 abc	74 abcdef	116 bcdef	56 bcde
60	8 bc	266 bcd	10 bc	60 bcdef	101 def	48 de
120	10 bc	260 bcd	12 bc	65 bcdef	109 def	50 de
240	20 abc	348 ab	15 abc	88 abcd	148 abc	65 bc
360	15 abc	297 abc	12 bc	77 abcde	142 abc	63 bcd
480	14 abc	298 abc	11 bc	72 abcdef	134 abcde	59 bcde
600	14 abc	207 cd	10 bc	59 cdef	111 cdef	49 de

^a Mean values within a column sharing the same letter are not significantly different based on Duncan's multiple range test at the 5% level of significance.

Table 6

Tocopherol (ppm), tocotrienol (ppm) and phytosterol (ppm) content of lipid fractions obtained after hexane extraction of steam-distilled fennel seeds.

	Control	15 min	30 min	60 min	120 min	240 min	360 min	480 min	600 min
Tocopherols									
α	9.8	6.5	9.4	6.5	7.6	10.1	10.7	16.3	12.0
γ	3.1	3.4	3.0	3.2	3.1	3.2	3.1	3.6	3.0
Tocotrienols									
α	46.4	56.9	62.1	47.9	48.5	41.9	34.9	38.1	27.5
γ	244.9	282.7	306.7	272.7	266.9	255.0	246.8	283.8	242.7
δ	5.3	6.0	6.1	6.0	5.2	4.9	4.7	6.0	12.0
Phytosterols									
Ave	1284	1413	1393	1362	1455	1471	1478	1521	1277
Cmp	676.3	731.4	724.7	726.7	741.4	787.0	781.6	848.1	772.8
Cyc	920.2	866.5	842.9	901.2	936.9	991.6	1029	1058	958.6
Met	173.9	144.3	142.4	165.4	179.1	176.1	177.5	183.1	165.4
Stan	172.1	1337	1422	1008	600.8	213.5	207.1	207.1	173.0
Ster	1422	182.1	192.3	159.2	921.4	1502	1623	1702	1580
Squ	57.1	149.3	122.1	139.8	89.8	116.0	98.3	120.5	125.9
Stg	1634	1650	1561	1663	1688	1741	1877	1943	1823

Ave = Δ 5-avenasterol; Cmp = campesterol; Cyc = cycloartenol; Met = 24-methylenecycloartanol; Stan = sitostanol; Ster = β -sitosterol; Squ = squalene; Stg = stigmasterol.

Steam distillation had little discernible effect on individual heteroatom content in FSO, as statistically equivalent concentrations of S, P, Na, K, and Ca were obtained for both the control and DT = 600 min samples. A statistically significant decrease in Mg content was noted for the control (80 ppm) versus the DT = 15–600 min samples (48–65 ppm). However, combined heteroatoms (Table 3) were generally lower for samples subjected to SD (450–686 ppm, combined) relative to the control (700 ppm). Among the steam distilled samples, combined heteroatom content behaved randomly as a function of DT.

Similar to the results obtained for heteroatoms, SD had minimal or random impact on individual tocopherol, tocotrienol, and phytosterol concentrations. Tocopherols identified in FSO included the α-and γ-homologues, and ranged from 6.5–16.4 ppm in the case of α-tocopherol and 3.0–3.6 ppm for γ-tocopherol. Tocotrienols were detected in generally higher amounts, which included the α-(27.5–62.1 ppm), γ-(242.7–306.7 ppm), and δ-(4.7–12.0 ppm) homologues. Several phytosterols were also identified in FSO, which included Δ 5-avenasterol, campesterol, cycloartenol, 24-methylenecycloartanol, sitostanol, β -sitosterol, squalene, and stigmasterol. β -Sitosterol, cycloartenol, and stigmasterol were the most abundant constituents detected, ranging from 159.2 to 1623 ppm in the case of β -sitosterol, 842.9–1058 ppm for cycloartenol, and 1561–1943 ppm for stigmasterol. The least abundant constituent was squalene, which ranged in concentration from 57.1 to 149.3 ppm. The combined concentration of phytosterols ranged from 6125 (DT = 60 min) to 7582 (480 min) ppm. SD was expected to have minimal impact on concentration of tocopherols, tocotrienols, and phytosterols due to their high bps.

3.6. Effect of DT on lipid quality

The properties of FSO are displayed in Table 7 and include AV, PV, IP, PP, KV (40 °C), density (25 °C), and Gardner color. All samples, including the control, were dark in appearance, as indicated by Gardner colors in excess of 18 (1 is lightest whereas 18 is darkest). Additionally, all samples exhibited IPs in excess of 24 h, thus indicating their stability to oxidation. For comparison, minimum limits for oxidative stability prescribed in the biodiesel standards are 3 (ASTM D6751) and 6 h (EN 14214) (Moser and Vaughn, 2010, 2012). We speculate that the high stabilities were due to the presence of FEOs, tocopherols, tocotrienols, and phytosterols.

Density (25 °C) and KV (40 °C) were both correlated to FEO content in FSO as a function of DT. Specifically, statistically significant higher KVs and lower densities were observed with increasing DT. As mentioned previously, longer DTs resulted in lower FEO contents in FSO. FEOs have higher densities and lower KVs than lipids, thus the effect of lower FEO content in FSO (i.e., longer DT) was to reduce density and increase KV. For example, the density of estragole, the predominant species detected in FEO, is 0.946 g/mL (Lide, 2008).

DT impacted AV, PV and PP of FSO, as significantly higher AVs and PPs were noted as DT increased. Such a result was not surprising, as SD subjected the sample to both elevated temperature and water, which are known to promote oxidative degradation and hydrolysis. Hydrolysis of lipids leads to higher AVs whereas oxidative degradation produces peroxide intermediates which cause PV to increase. Although PV increased as DT increased, the increases were not sufficient to yield IPs below 24 h. Acids have higher melting points than the corresponding esters, so it was no surprise that PP increased with increasing AV and DT.

Table 7

Properties of lipid fractions obtained after hexane extraction of steam-distilled fennel seeds.^a

DT (min)	AV (mg KOH/g)	PV	IP (h)	PP (°C)	KV (mm ² s)	Gardner color	Density, 25 °C (g/mL)
Control	5.65 a	16.0 a	>24	-19.4 a	25.47 a	>18	0.919 a
15	5.93 ab	18.4 ab	>24	-16.9 c	25.93 ab	>18	0.918 ab
30	6.27 b	18.4 ab	>24	-16.7 c	25.62 a	>18	0.918 ab
60	6.31 b	21.2 bc	>24	-17.4 bc	25.88 ab	>18	0.918 ab
120	6.56 b	19.6 abc	>24	-16.3 cd	26.86 b	>18	0.918 ab
240	7.63 c	28.8 d	>24	-18.9 a	29.84 c	>18	0.918 ab
360	7.53 c	28.8 cd	>24	-18.7 ab	31.07 d	>18	0.917 b
480	7.33 c	27.2 d	>24	-15.2 d	32.79 e	>18	0.917 b
600	7.58 c	32.6 d	>24	-15.0 d	33.97 f	>18	0.915 c

^a Mean values within a column sharing the same letter are not significantly different based on Duncan's multiple range test at the 5% level of significance.

4. Conclusions

DT had a statistically significant effect on yield of FEO from fennel seeds, with longer DTs resulting in higher yields. The major constituents identified in FEO were estragole, fenchone, limonene, and anethole. The most abundant species was estragole. The concentrations of anethole and estragole tended to decrease with increasing DT whereas limonene increased and fenchone was essentially unaffected. The species most affected by DT were those with the lowest bps, such as limonene. Although the concentrations of the EO constituents generally changed with DT, antioxidant capacity was unaffected at DTs ranging from 15 to 960 min.

The yield of lipids extracted from steam distilled fennel seeds was unaffected by DT and ranged from 21.7 to 22.8 mass%. The fatty acid composition was also unaffected by DT, and the major constituents were petroselenic (67.0–71.3%) and oleic (12.0–16.4%) acids. The concentrations of tocopherols, tocotrienols, and phytosterols were unaffected by DT whereas unsaponifiables and EO content in lipids decreased with increasing DT. Heteroatom content and Gardner color were unaffected by DT. Subjection of resident lipids to SD resulted in partial hydrolysis, which was manifested by increased AV relative to lipids extracted from non-distilled seeds. The increased content of free fatty acids in lipids as a result of hydrolysis also negatively impacted KV and PP. The elevated temperatures associated with SD promoted peroxidation, as measured by increased PVs with increasing DT. However, the extent of peroxidation was not sufficient to cause reduced IPs, as IP was unaffected by DT.

The concentrations of soluble nutrients in steam-distilled, defatted fennel seed meal decreased or tended to decrease with increasing DT. Correspondingly, the concentrations of insoluble matter increased with increasing DT. As a result, *in vitro* DM degradability decreased with DTs > 120 min.

In summary, DT impacted feed quality of steam-distilled, defatted seed meal and lipid quality but did not affect EO composition and antioxidant capacity. However, the negative impact of SD on lipid quality was not sufficient to render it unacceptable as a feedstock for applications such as biodiesel.

Acknowledgements

Julie Anderson, Kim Ascherl, Benetria Banks, Billy Deadmond, Jeff Forrester, and Kathy Rennick (USDA-ARS-NCAUR) are acknowledged for technical assistance.

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